Phytoplankton Imaging and Analysis System: Instrumentation for Field and Laboratory Acquisition, Analysis and WWW/LAN-Based Sharing of Marine Phytoplankton Data (DURIP)

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LONG TERM GOALS

Our long term goal is to understand the ecology of phytoplankton, especially the large, colonial diatoms which frequently dominate the flora of coastal shelves, upwelling areas, fjords and banks. We are interested in ways in which species-specific properties, including cell and colony size and shape interact with physical mixing processes to regulate the spatio-temporal distribution of diatoms. We wish to understand these processes in sufficient detail to be able to predict bloom dynamics, size structure, and the impact of species-specific characteristics of the phytoplankton on ocean optics.

OBJECTIVES

This award addresses that goal by providing instrumentation and computer systems which facilitate the processes of: (1) acquiring images of the different kinds of plankton which are present in our samples, (2) electronically sharing those images (data) via the world-wide-web (WWW), or local networks (LAN), (3) quantifying size, shape, and other parameters of plankton cells and colonies via image analysis and image reconstruction.

APPROACH

The components of the Plankton Imaging and Analysis Laboratory (PIAL) have been selected to provide us with a series of new capabilities for both laboratory and field work:

(1) <u>Microscopes</u>. Our microscopy facilities combine the best offerings of the Nikon and Zeiss instrument lines. The Nikon system is equipped with phase contrast, brightfield, Nomarski Differential Interference Contrast and Epifluorescent optics, primarily for use in the lab. Nikon provides superb ergonomics, and ease of use for novice users (students). However, while Nikon's phase contrast is commendable in black and white, it is significantly compromised in color. Carl Zeiss provides what I believe to be the best phase contrast optics commercially available. The Zeiss microscope is equipped with phase contrast, and limited Nomarski

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- capabilities. It will be used extensively in the field. The Zeiss Akioskop 2 is also equipped with a motorized focus mechanism, which can be interfaced with a computer to allow optical serial sectioning of specimens. A "z-stack" of serial sections can be recombined to create a single, infocus image of a highly three-dimensional specimen. An extension of this approach can compose full, 3-D reconstructions of specimens.
- (2) <u>Digital documentation of plankton</u>. Standard, film-based imaging requires exposure of the film (usually an entire roll), commercial processing (for color film), and subsequent scanning of prints or slides into the computer. Digital imaging systems bypass most of these steps, and allow direct processing of single, or multiple images. Much time is saved, because all stages of the work take place in our own facilities.
- (3) <u>Video</u>. Many phytoplankton are motile. It is not possible to attain an appreciation of the effect this feature can have on their life-histories, and spatio-temporal distribution in the sea from still images. The Optronics video systems allow us to record live, video images of motile taxa in standard S-video, RGB or digital format. Adobe Premiere software can be used to produce illustrative or educational clips, which can be distributed via CD, or via the web.
- (4) <u>Film-based imaging</u>. Digital imaging offers tremendous advantages for flexibility and speed of processing, but there remain applications for which the superior resolution of film is the best medium. For this reason, the system includes Nikon's automatic 35 mm camera system, which will allow us to take high resolution photographs when needed to answer specific questions.
- (5) <u>Image Analysis</u>. Current capabilities include Zeiss' Axiovision, and NIH Image.
- (6) <u>Computers</u>. The facility uses both MacIntosh and PC platforms. Zeiss computerized microscope software and imaging runs on a Dell Pentium III, 600 mHz machine. Sony digital video tape recording equipment will run into a Power Macintosh G4, which has not yet been delivered.
- (7) <u>Communication/data sharing</u>. A variety of hardware and software systems streamline the process of acquiring images, and placing them on the web. In future field experiments, we will be able to utilize a wireless network, such as the one implemented by Cowles in East Sound (1998), to relay images of plankton to our colleagues, to a classroom, or to interested parties in other location in near-real time.

Further information on specific components which make up this facility, and on their technical capabilities can be found on the PIAL web site (http://thalassa.gso.uri.edu/pial).

WORK COMPLETED

Work this year has focused on acquiring instrumentation and constructing the facility. The Plankton Imaging and Analysis Laboratory (PIAL) consists of numerous components, which function together to provide new capabilities to our research team. These include microscopes, computer systems, digital and 35 mm camera systems, digital video recording equipment and image processing, image analysis, digital video editing and web authoring software. Most items are commercially available, but both Nikon and Zeiss have made, or are in the process of developing custom modifications to meet our specifications. Most instrumentation is in place, but a few custom items, as well as newly released instrumentation (e.g. Macintosh's new G4 computers) are still outstanding.

RESULTS

This facility is very new — there has been little time to conduct research. This new equipment offers us a whole new world of capabilities, and is one of the most exciting events of my career. As we develop new plankton visualization methodologies, we will post examples on the web site.

IMPACT/APPLICATIONS

In addition to expanding our oceanographic research capabilities, this equipment serves an important educational function because it facilitates the sharing of visual, species-specific information about plankton with several different audiences. As a generalization, familiarity with phytoplankton at the species-specific level has traditionally been limited to taxonomists and ecologists. It is usually published in a highly specialized literature, which is read mainly by other taxonomists. However, I have found that researchers in many other areas are very interested in the properties of phytoplankton when they can easily access the information. This is best accomplished using a direct, visual approach — pictures. Use of the web as a vehicle for dissemination of information allows anyone who is interested to find it via search engines.

We are working to develop 3-D reconstruction techniques will allow us to visualize microscopic plankton in 3-D, and render the object/shape on the computer. Quantitative data describing the 3-D shape of an organism may allow us to interact with both optical oceanographers, and Navy systems developers in new ways.

TRANSITIONS

- (1) This equipment will facilitate the development of the phytoplankton web sites developed in conjunction with ONR and NSF sponsored research. These sites have proven very useful in distributing information on phytoplankton to a large and diverse community of interested people.
- (2) Researchers at NUWC (Newport, R.I.) have expressed interest in collaborating to obtain data on the size and shape of phytoplankton present in the water column during NUWC instrument tests at a site off the Coast of California.

RELATED PROJECTS

This equipment provides us with capabilities which will allow us to interact with other efforts. For example, in future field efforts, this equipment will allow us to utilize a wireless network such as that constructed by Cowles (DURIP N00014-97-1-0349) to rapidly share visual information on phytoplankton. It will facilitate our interaction with other Thin Layers PIs who are interested in species-specific information, for example Donaghay, Holliday, Weidemann, Zaneveld, Alldredge, Perry, Cowles, Gifford.



Chaetoceros concavicornis, a diatom from East Sound